III. MATERIALS AND METHODS

Investigations on both nematodes and plants were conducted at ambient temperature and humidity conditions in green house and under semiaseptic conditions of potted soil in Nematology Laboratory of Central Rice Research Institute, Cuttack.

Live nematodes viz., Pratylenchus zeae were collected from roots of maize var. Vijaya from the agricultural farm, OUAT, Bhubaneswar and Meloidogyne graminicola from roots of rice var. Annapurna from CRRI farm, Cuttack. Pure cultures of these nematodes were maintained in sterilized soils fertilized with nutrients dose of N_{60}, P_{30} and K_{30} in pots on rice var. Annapurna for studies as this cultivar was found to be susceptible to Pratylenchus sp. and M. graminicola (Prasad,1977; Sahu and Chawla, 1988).

For experimental purposes, motile P. zeae were extracted from soils of the pure culture stock by wet seiving methods (Cobb, 1918) and then processed through tissue paper assembly (Schiendler, 1961). Root endoparasites were extracted following Young (1954). Second stage juveniles of M. graminicola were collected by incubating egg masses on tissue paper (Young, 1954).

Experimental inoculations were made with all stages of P. zeae (Roman and Hirschmann, 1969) unless otherwise

stated. Prior to inoculations, nematodes were surface sterilized with 0.02% (0.2 mg/ml) mercurochrome for 1 hr and then washed in sterile water (Tiner, 1961 a,b).

Root inoculations were made by exposing the feeder roots and pouring the nematode suspension as near as possible to the feeder roots.

The egg and nematode population inside roots were counted by staining the roots in acid fuschin lactophenol (Franklin and Goodey, 1944). Hence, any mention of root counts will imply a prior staining of the roots.

The above said basic procedures were followed in the following experiments.

1. MORPHOLOGY AND MORPHOMETRICS

Live larvae and adult P. zeae were fixed in hot formalin and processed for permanent mounts following Seinhorst (1959, 1966). Twentyfive each of fixed juveniles and adults were taken for morphometrical studies (De Man, 1980). The morphology of P. zeae was compared with the descriptions of Graham (1951), Atkins et al. (1955), Yokoo (1962), Nath et al. (1975) and Fortuner (1976) and the morphometrics with those of Graham (1951) and Nath et al. (1975).

Data on the morphometrics of juveniles and adult stages of P. zeae were used to compute the growth of the nematode and the biomass of each stage was estimated by the

formula of Andrassey (1956).

2. ROOT INVASION

Single seedling of rice var. Annapurna was raised in plastic cups filled with 100 g of sterilized soil. Five day-old seedlings were inoculated with 25 each of J_2, J_3, J_4 and adult stages of P. zeae in 4 cups in four replicates. Seedlings were uprooted from each replicate on 2nd, 6th and 10th days after inoculation and the number of P. zeae inside root was counted. The percentage of inoculum that successfully entered roots was compared in respect of each stage of the nematode.

3. LIFE HISTORY

A. Post-embryonic development

Single seedling of rice var. Annapurna was raised in earthen pots each containing 250 g of sterilized soil. Five days after germination each seedling was inoculated with 20 freshly hatched 2nd stage juveniles (Rao and Israel, 1973). In all, 60 seedlings were inoculated. Three seedlings were uprooted on the 2nd, 3rd and on alternate days thereafter till the 37th day and the number of endoparasitic stages was counted. The process continued till hatching of the freshly laid eggs. Each stage of nematode was identified on the basis of earlier descriptions by Graham (1951) and Nath et al. (1975). The duration of each stage of development was

computed as the period between two successive stages (Prasad, 1977).

B. **Fecundity**

Seedlings of rice var. Annapurna were raised in plastic cups filled with 100 g of sterilized soil at the rate of one in each cup. Five days after germination each seedling was inoculated with 25 gravid female P. zeae. Four days after inoculation all seedlings were uprooted. Eggs laid inside the root was counted. All other uprooted seedlings were planted in freshly sterilized soil to count the number of eggs laid each day till the 8th day by uprooting 3 seedlings daily.

4. **PATHOGENICITY**

Single seedlings of rice var. annapurna was raised in 15 cm earthen pots each filled with 2 kg of sterilized soil. Ten days after germination seedlings were inoculated at the logarithimic series of 10, 10^2, 10^3 and 10^4 nematodes per seedlings (Nath et al., 1978; Azmi, 1984). Each treatment was replicated thrice with uninoculated plant as control. Plants were harvested 60 days after inoculation and length and dry weight of shoots and roots were recorded. Stained roots were examined for studying damage syndromes and counting endoparasitic population.

5. CONCOMITTANT PATHOGENICITY

Concomittant pathogenicity of P. zeae and M. graminicola was studied on rice var. Annapurna by inoculating 10 days old seedlings in pots filled with 2 kg of sterilized soil. Single inoculation of freshly hatched larvae of M. graminicola at the rate of 10, 10^2, 10^3 and 10^4 and combined inoculation at the rate of 1:1(P. zeae and M. graminicola)to a final inoculum level of 10, 10^2, 10^3 and 10^4 were given per seedling (Upadhyaya and Swarup, 1981). Height and dry weight of shoot and root were taken 60 days after inoculation and the endoparasitic population of both nematodes and root knots number per plant were counted from roots.

The build up index i.e. population at a time t/initial population (P^f/Pi) was compared in the inoculations in respect of the individual nematode at each inoculum level. Since the inoculum density was on the log scale viz., (50) 0.7 to (10000) 4.0, the final population after 60 days were also transformed to log values to determine if the growth was exponential. Regression analysis was made between inoculum density and final population density in the three series of inoculations to estimate the expected populations of each nematode $Ye = (\overline{Y} - b\overline{X}) + bX$, where Y was the mean final popuation, X was the mean inoculum level and 'b' the regression coefficient (Goulden, 1960).

6. HOST RANGE

Recording the presence of P. zeae on a plant (Table 1) does not always confer its status as host. To define the host status of a species it was desired to inoculate different crop plants used in rice based agriculture or plants found associated in rice fields.

Seventeen crops, twenty weeds and eighteen wild rices (Tables 10A, 10B and 11) were raised singly in earthen pots filled with 1 kg steam sterilized soil. Inoculation at the rate of 150 P. zeae per plant were given one week after germination in crops and one week after transplantation of weeds and wild rices. Each treatment was replicated thrice with rice var. Annapurna as a check. Thirtyfive days after nematode inoculation the reaction of the plants was determined by estimating nematode population per g root (Mani and Reddy, 1986).

7. MANAGEMENT AND CONTROL

A. Effect of organic nematicides

Single seedling of rice var. Annapurna was raised in earthen pots filled with 2 kg of sterilized soil. Seven days after germination pre-inoculation application (Kaul and Sethi, 1987; Meher, 1991) of 3 organophosphates viz., ebuphos 10 G (S'S-di-sec-butyl 0-ethyle phosphorodithioate),

phorate 10 G (0-0-diethyl-S-ethyle thiomethyl phosphoro-dithioate), ethoprop 4 G (0-ethyle-SS-dipropyl phosphoro-dithioate), and 4 carbamates viz., carbofuran 3 G (2-3 dihydro-2-2-dimethyl-7-benzofuranyl methyl carbamate), carbosulfan 25 DS (2-3 dihydro-2-2 dimethyl-7-benzofuranyl (dibutyl amino-thio)methyl carbamate), isoprocarb 4 G (2-isopropyl-phenyl-N-methyl carbamate), and patap 4 G (SS' (2 dimethyl aminonitromethylene) Bis-thiocarbamate) were given in three different doses of 0.5, 1.0 and 2.0 kg ai.i./ha to soil. Proper moisture condition in soil was maintained and three days after nematicidal application, P. zeae were inoculated at the rate of 300 nematodes/ seedling. Plants were uprooted 35 days after inoculation. Height and dry weight of shoots and roots were measured and nematode population inside roots was counted. Nematode mortality, plant growth and final population were recorded.

B. **Effect of botanicals (oil cakes)**

i. **In vitro tests**

Aqueous extracts of 8 oil cakes viz., cotton (Gossipium sp.), groundnut (Arachis hypogea), karanj (Pongamia glabra), mustard (Brassica campestris), mahua (Madhuka indica), neem (Azadirachta indica), polang (Callo-phyllum inophyllum) and til (Sesamum indicum) were prepared by soaking 5 g each of pulverised cake in 25 ml distilled

water at room temperature for 48 hr. Each slurry was then filtered through a fine nylon mesh and the filtrate was stored in refrigerator as 100% concentration of the extract. Further dilutions to 50%, 25%, 12.5% and 6.25% were made with water. Concentrations of each extracts were dispensed in cavity blocks at the rate of 2 ml for three different periods of exposures. Twentyfive live P. zeae, collected from pure cultures, were released in each cavity block. The experiment was replicated thrice for each concentration. At intervals of 12, 24 and 48 hr after release of P. zeae, the nematodes were transferred to water and the mortality was recorded after 12 hr in water. The percentage mortality in the treatments were compared to study the contact toxicity of extracts.

Correlation and regression analysis of concentrations (X) and mortality percentage (Y) at 48 hr was attempted with the data on the toxicology of oil cake extracts (Goulden, 1952). LD_{90} values from the equations were calculated and compared with those obtained from concentration mortality curves. Fiducial limits, lower $f(L_1)$ and upper (L_2) were calculated from the slope (Bxy) and t (0.05) values for 4 degrees of freedom which were given by L_1 = Slope - t value x standard error and L_2 = Slope + t value x standard error (Heinrichs et al., 1981).

ii. **Seedling root dip treatment**

Aqueous extracts of these eight oil cakes, tested in the contact toxicity assay, were used as pre-planting root dip of seedlings var. Annapurna. One week old seedlings were used for root dipping for 8 hr in five concentrations (6.25% to 100%) of the extracts after which the seedlings were transplanted at the rate of one per pot filled with 0.5 kg of sterilized soil. The experiment was replicated thrice with untreated seedling as control. Each seedling was inoculated with 100 P. zeae. Plants were uprooted on 15th day of inoculation and the endoparasite population in roots was estimated. The percentage reduction in P. zeae population in each treatment over control was calculated and compared with different concentrations of the extracts.

C. **Effect of oil cakes and nematicides as soil amendments**

Oil cakes viz., neem, karanj, mustard, mahua, polanga, groundnut, til and cotton were pulverised and added to sterilized soil filled in earthen pots(2 kg in each) at a dose of 1 ton/ha. Field moisture condition was maintained to allow decomposition of oil cakes for 15 days. To another set of pots filled with sterilied soil, phorate and carbofuran were applied at the rate of 1 kg a.i./ha. Each treatment was replicated thrice with untreated soil as control.

Seven days old seedlings of rice var. Annapurna were planted at the rate of one in each of the above pots. On the 3rd day after planting each seedling was inoculated with 500 P. zeae (Bhattacharya and Goswami, 1987; Goswami and Vijaylaxmi, 1987). Plants were harvested at 35 days after inoculation and number of tillers per plant, height and fresh weight of shoots were recorded. Population of nematode in roots was determined. Data were analysed to compare the relative effectiveness of the treatments.