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Incidence of Nematode Diseases in Makhana and their Possible Control in Katihar District of North Bihar

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Abstract: ***Euryale ferox Salisb. commonly known as makhana is an important aquatic annual seed propagating cash crop in shallow water bodies in north Bihar and lower Assam regions of India. On account of being fatless, aphrodisiac, spermatogenic and with high carbohydrate and protein contents, makhana is in high demand in western and gulf countries. Plant parasitic nematodes causes a considerable damage to agricultural crops. Rhizospheres of makhana plant arealso infected by several nematodes which cause a significant loss in crop production. Very little information is available on the parasitic nematodes of makhana crop. In a nematological study conductedin Katihar district of North Bihar altogether 6 species of nematodes belonging to 6 genera associated with rhizospheres of makhana were identified. Hirschmanniella oryzae were the most abundant species dominatingother nematodes in all the months and seasonswithan average of 238 per 100g soil. Maximum population density of nematodes was recorded during September to October and minimum during January to February. In reducing the population of nematodes use of different plant leaves such as Azadirachtaindica, Carica papaya, Thevetia peruviana,Tinospora cordifolia and Ricinus communis were found effective in makhana cultivated ponds. Herbal leaves were found to be more effective in controlling nematode population as compared to nematicide which in turn affects the production of fish in integrated culture.***

Keywords: Makhanarhizosphere, parasitic nematodes, loss, crop yield, herbal control

1. Introduction

*Euryale ferox*Salisb. (makhana /gorgoan nut/fox nut) is a rooted macro-hydrophyte grown commonly as a cash crop in the wetlands of Northern Bihar [1]. Makhana refers to yummy pops or white puff balls prepared through mechanical smacking of the baked seeds (guria) of *E. ferox*. It belongs to the family Nymphaeaeae and grows naturally in wild form or cultivated in ponds, low land stagnant water etc. The plant is widely prevalent in tropical and subtropical regions accomplished with humid to subhumid environment and mainly cultivated as a source of starch and protein [2, 3]. India is the largest producer of makhana in the world and Bihar state alone produces up to 90% of total makhana cultivation in india [4]. This heavy makhana production is particularly due to plenty of wetlands *viz*. ponds, lakes, stagnant water bodies etc. in northern Bihar made especially from meandering Koshi and Mahananda rivers [5]. Cultivation of makhana is possible in general agricultural fields also with clay soil for better water holding capacity [6]. Makhana thrives best in a range of airtemperature 20^0-35^0C, relative humidity 50-90% and annual rainfall about 100-250 cm[7]. Morphologically makhana plant consists of a short, thick and fibrous root; underground and rhizomatous stem; several orbicular floating glabrous green or pinkish leaves with prickly petiole; violet-blue or red cleistogomous (common, 70-80%) and chasmogomous flowers and spongy prickly round fruits usually containing 20-40 black arillate thickly encrusted heathed seeds [8]. The seeds are locally known as guria. Several species of nematodes have been reported around the rhizosphere of gorgonnut in both eutrophic and mesotrophic ponds throughout the world in which some are of parasitic forms. Recently there has been a growing realization of the importance of nematodes worldwide due to loss caused by them in agricultural crops [9]. More than 4100 species of phytoparasitic nematodes are responsible for an estimated economic loss in agriculture sector of nearly $ 125 billion annually [10]. Many of which are microscopic and free living. Out of 1 million estimated species of nematodes [11] about 25,000 nematode species have been described **[**12, 13]) of which more than 50% are parasitic on most plants and animals including humans [11, 14]). Free living forms of nematodes live in water and soil. 90% of nematodes reside in top 15cm of soil and they play an important role in the nitrogen cycle by the way of nitrogen mineralization [15]). They have been successfully adapted to all types of ecosystems from marine to freshwater, to soils, and from Polar Regions to the tropics as well as highest to the lowest of elevations. They are ubiquitous in freshwater, marine and terrestrial environments where they outnumber other animals in both individual and species count with a wide variety of shapes, sizes (0.3mm to 78m) and structures. They also occur at the bottom of ponds, lakes, rivers and at enormous depths in the oceans and in all types of soil [16, 17].

Loss in agricultural crops due to plant parasitic nematodes has attracted the attentions of agriculture scientists and biologists worldwide. Several species of nematodes have also been reported around the rhizospheres of gorgon nut (*Euryale ferox* Salisb.) in both eutrophic and mesotrophic ponds throughout the world in which some are parasitic and others free living. Cultivators of Katihar district are the major producers of makhana in north Bihar and India. Hence, it was thought necessary to throw light on the association of nematodes with makhana in the soils of different ponds of Katihar district. The objective of the present study was to identify plant parasitic nematodes associated with rhizosphere, soil and root samples of makhana plant. Also, the study was designated to provide more extensive information on the distribution of plant parasitic nematode species, and document their presence and abundance to estimate the level of infestation of each species that may have significant impact on agriculture and their control in Katihar district of North Bihar.

2. Materials and Methods

Nematological study was carried out in different blocks of Katihar district of North Bihar from 2017- 2019 from different ponds of different villages (Table - 1). A total of 90 soil and root samples were collected from 9 pondsof surveyed area. Composite soil and root samples of 1 kg each were collected from makhana rhizosphere of the surveyed pond. Each soil sample was thoroughly mixed and 100 cm^2 soil was washed for nematode extraction by means of Cobb's wet-sieving and centrifugal sugar floatation techniques [18]. Roots were washed free of soil and examined for root galling and root-knot nematode infection. Adult females of root-knot nematodes were isolated from the infected galled roots. Identification of root-knot nematodes to the species level was done by preparing and examining the perineal patterns of isolated adult females [19]. Nematode identification was based on the morphology of adult and larval forms [20, 21, 22, 23, 24]. Plant parasitic nematode were counted using Peter's ml eelworm counting slide under a compound microscope. Different dried leaves of plant (40kg/ ha) were used on nematode population in makhana cultivated ponds.

Table 1: Study Area of Katihar District

Site	*Block*	*Villages*
I	BARARI	(i)BishariaPokhar (ii)Ronia Railway Dhala Pond (iii)Laxmipur Road Pond
II	KORHA	(i) GorgammaDhar / Pond (ii) NajraChowki Pond (iii) PulbariaChowk Pond
III	KATIHAR	(i) Mania Pond (ii) Kolasi Pond (iii) Nayatola Pond

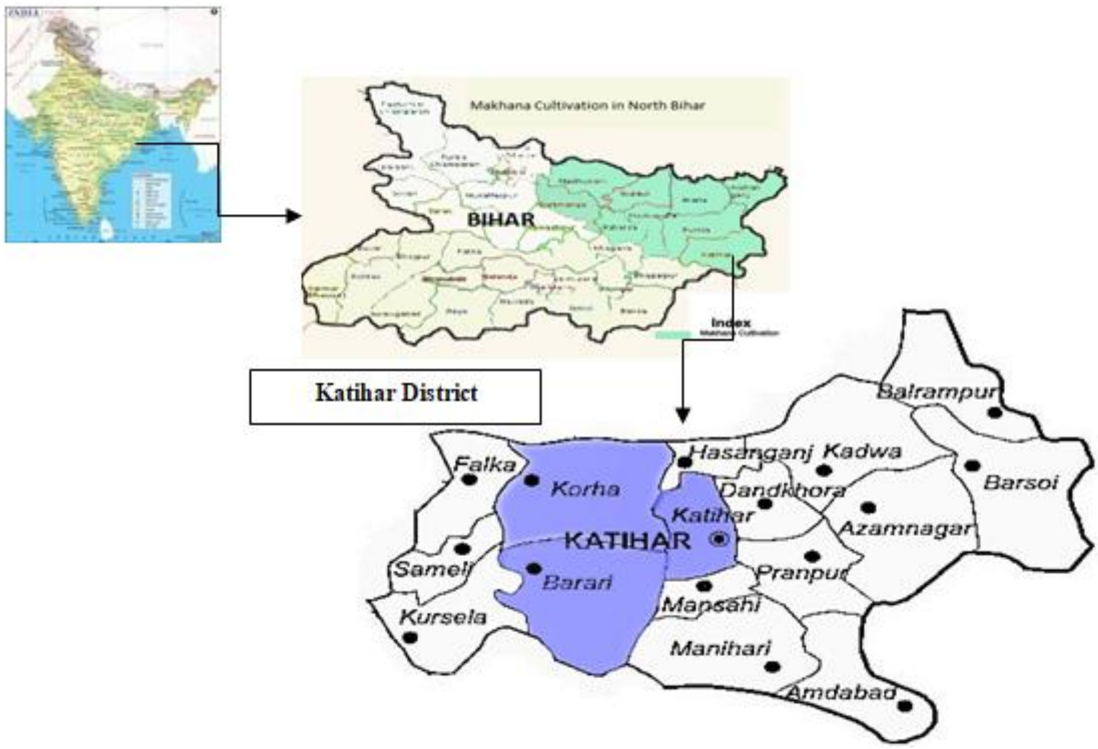


Figure 1: Location Map of Katihar District in Bihar

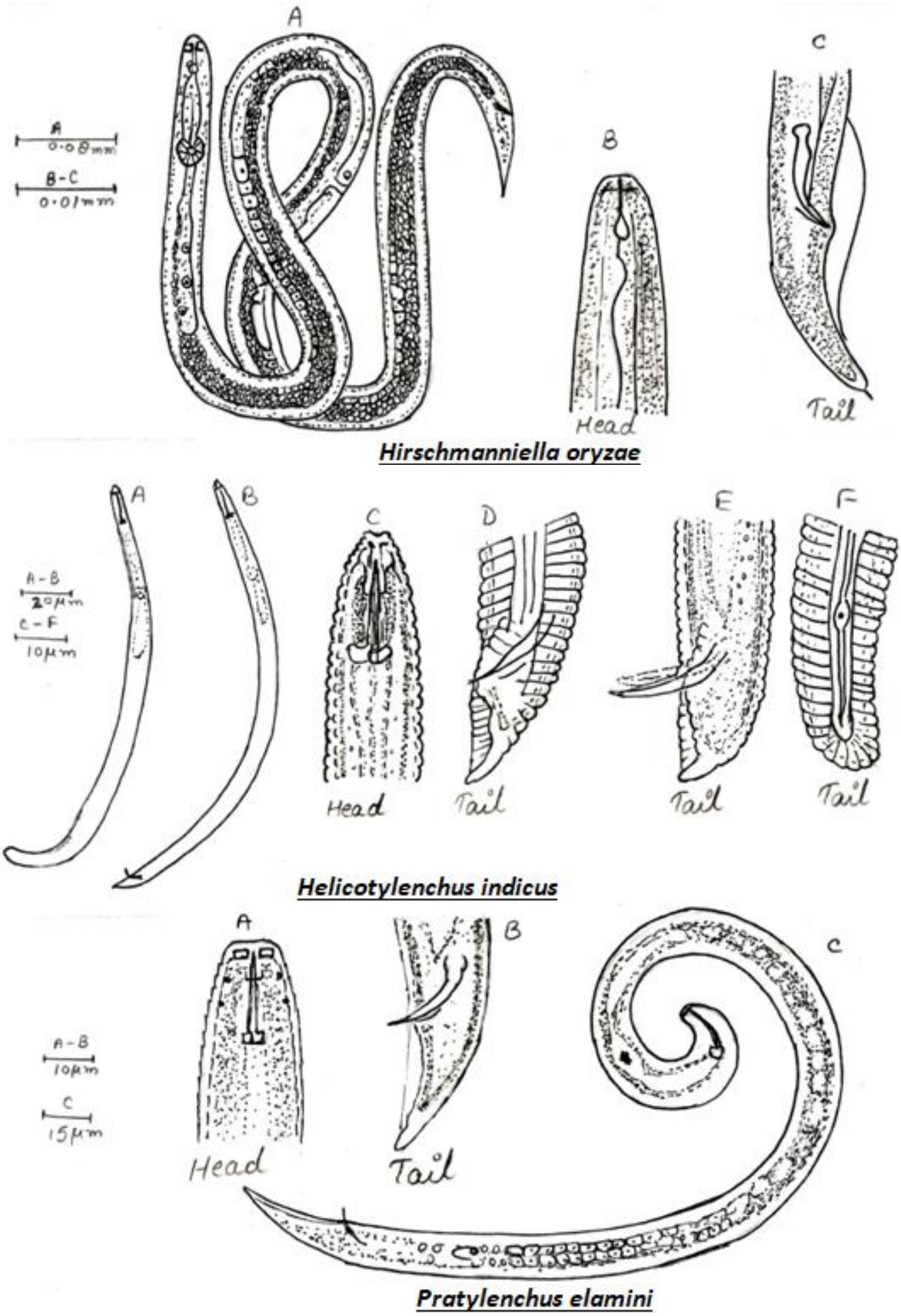
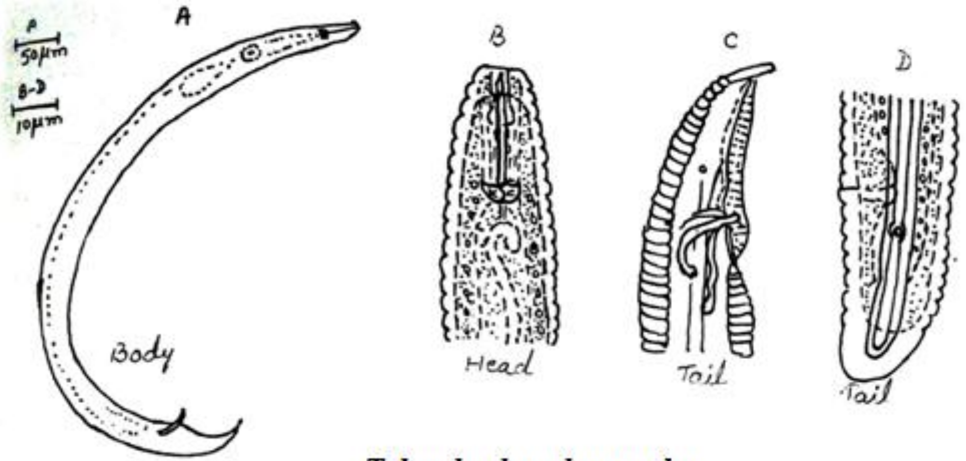
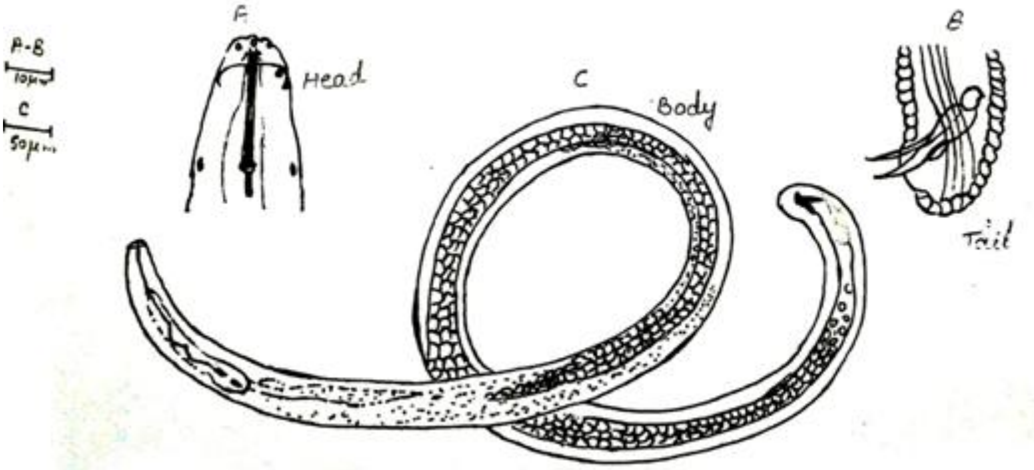


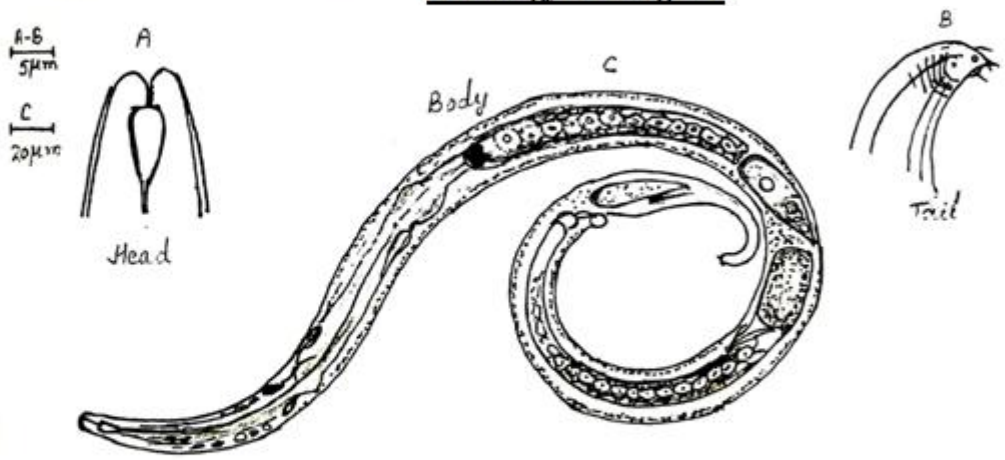
Plate 1: Nematodes Associated with Makhana Rhizosphere in Katihar District



Tylenchorhynchus nudus



Meloidogyne incognita



Chronogaster gracilis

Plate 2: Nematodes Associated with Makhana Rhizosphere in Katihar District

3. Results

Nematodes encountened around rhizosphere ofmakhana in different ponds of Katihar district are mentioned in **Table- 2.** Data present that ponds of Site-I and site-III refer an example of eutrophic ponds whereas ponds of Site-II refer an example of mesotrophic pond. In the present study a total of 6 species belonging to 6 genera of nematodes were identified from the soil collected around the rhizospheres of Makhana(**Table - 7.1, Plates - 7.1, 7.2**). Out of 6 genera, C*hronogaster gracilis* represents the non-parasitic forms where as *Helicotylenchus indicus, Hirschmanniella oryzae, Pratylenchus elamini, Meloidogyne incognita* and *Tylenchorhynchus nudus* are parasitic ones. *Chronagaster gracilis,H. indicus, H. oryzae* and *Pratylenchus elamini* were found in all the three sites where as *Tylenchorynchusnudus* was found at Site II only. Among these *H. oryzae, P. elamini* and *T. nudus* cause **root necrosis**, **lesion**and **dwarfing diseases** respectively in makhana plants.

Table : 2- 4 depict the population density (per 100 g of soil) of different nematodes (average of two years) around the rhizospheres of *makhana* and their monthly and seasonal fluctuations in the ponds of Katihar district. The number of *Chronogaster gracilis* at Site I ranged from 14-34, *Helicotylenchus indicus* 5-15, *Hirschmanniella oryzae* 72-240 and *Pratylenchus elamini* 7-42, *Meloidogyne incognita* 52-134 and *Tylenchorhynchus nudus* nil per 100g of pond soil. Site-II exhibited the presence of 12-68 *C. gracilis,* 7-30 *H. indicus,* 116-304 *H. oryzae,*10-52 *P. elamini, 62-168 M. incognita* and 9-30 *T. nudus*.Site III showed the minimum monthly variations of nematodes *C .gracilis* 7-32, *H. indicus* 2-10, *H. oryzae* 24-180, *P. elamini* 4-16, *M. incognita 12-72* and *T. nudus* nil.

Hirschmanniella oryzae dominated the other nematodes in all the months and seasons. Seasonal fluctuations showed the maximum number of nematode population (no. /100g soil) during rainy season 1431 at Site –II followed by 1027 at Site-I and 757 at Site-III. Lowest seasonal value was recorded during winter season (521 at Site-I, 928 at Site-II and 402 at site-III) however, summer showed the moderate values. Among months maximum population density was recorded during September, October and minimum during January to February. During June in summer the no. of nematodes at all the sites were reduced significantly

Effects of dried leaf powders (40 kg/ha) of different plants on nematode population in makhana ponds were also studied and depicted in **Table-5.** Leaf powder of neem (*Azadirachta indica*) was proved to be the most effective in decreasing the population of nematodes by 50.7% followed by Castor (*Ricinus communis)* 43.4%, giloy (*Tinospora cordifolia*) 35.8%, yellow kaner (*Thevetia peruviana)* 30% and papaya (*Carica papaya)* 23.1%.

Table 7.1: Nematodes Found Around Rhizospheres of Makhana (*Euryale ferox Sa*lisb.) in Different Ponds of Katihar District

Name of Nematode species	Forms	Disease caused	Occurrence		
			Site-I	Site-II	Site-III
1. Chronogaster gracilis Cobb.	Non-parasitic	-	+	+	+
2. Helicotylenchus indicus Siddiqi	Parasitic	-	+	+	+
3. Hirschmanniella oryzae	Parasitic	Root necrosis	+	+	+
4. Pratylenchus elamini	Parasitic	Lesion	+	+	+
5. Tylenchorhynchus nudus	Parasitic	Dwarfing	-	+	-
6. Meloidogyne incognita	Parasitic	Root Knot	+	+	+

Table 7.2: Average Population Density (per 100g Soil) Seaosnal Fluctuations of Different Nematodes Around Rhizospheresof Makhana and at Site –I

Crop stage	*Period*	*C. gracilis*	*H. indicus*	*H. oryzae*	*P. elamini*	*T. nudus*	*M. inconita*	*Total*
Seeding	Oct	24	20	240	28		134	446
	Nov	19	13	136	14		56	238
	Dec	17	8	84	9		52	170
	Avg	20	13.67	153.33	17		80.67	284.67
Seedling	Jan	15	5	72	8		26	126
	Feb	14	6	94	7		62	183
	Mar	23	6	132	14		75	250
	Avg	17.33	5.67	99.33	9.67		54.33	186.33
Grand Growth	Apr	22	7	156	18		84	287
	May	18	8	142	16		78	262
	Jun	16	5	108	10		64	203
	Avg	18.67	6.67	135.33	14.67		75.33	250.67
Post Harvest	Jul	22	10	146	23		87	288
	Aug	24	12	172	30		98	336
	Sep	34	15	188	42		105	384
	Avg	26.67	12.33	168.67	31.67		96.67	336

Table 7.3: Average Population Density (per 100g Soil) Seaosnal Fluctuations of Different Nematodes Around the Rhizospheresof Makhana at Site –II

Crop stage	Period	*C. gracilis*	*H. indicus*	*H. oryzae*	*P. elamini*	*T. nudus*	*M. incognita*	Total
Seeding	Oct	43	30	304	38	21	107	543
	Nov	40	20	252	26	14	158	510
	Dec	32	12	160	20	13	69	306
	Avg	38.33	21	238.67	28	16	111.33	453
Seedling	Jan	12	7	116	10	10	45	200
	Feb	15	9	154	18	9	74	279
	Mar	38	11	184	22	22	93	370
	Avg	21.67	9	151.33	16.67	13.67	70.67	283
Grand Growth	Apr	36	13	202	30	18	98	397
	May	33	18	176	23	14	84	348
	Jun	26	10	138	18	14	62	268
	Avg	31.67	13.67	172	23.67	15.33	81.33	337.67
Post Harvest	Jul	46	17	172	35	20	84	374

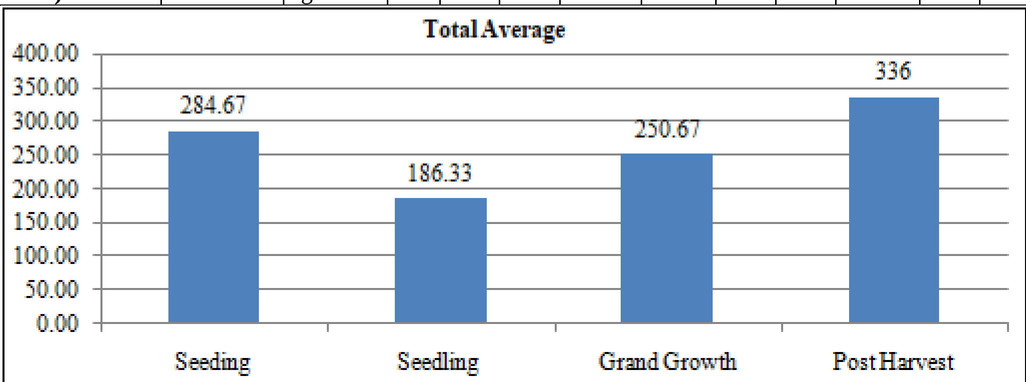
	Aug	57	22	216	52	28	126	501
	Sep	68	26	263	44	30	168	599
	Avg	57	21.67	217	43.67	26	126	491.33

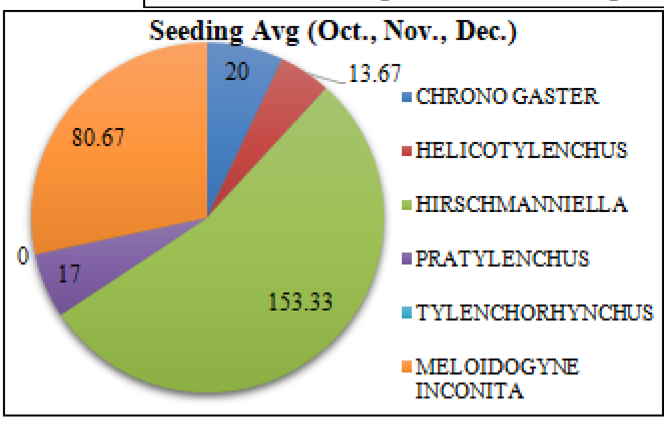
Table 7.4: Average Population Density (per 100g Soil) Seaosnal Fluctuations of Different Nematodes Around the Rhizospheres of Makhana and at Site –III

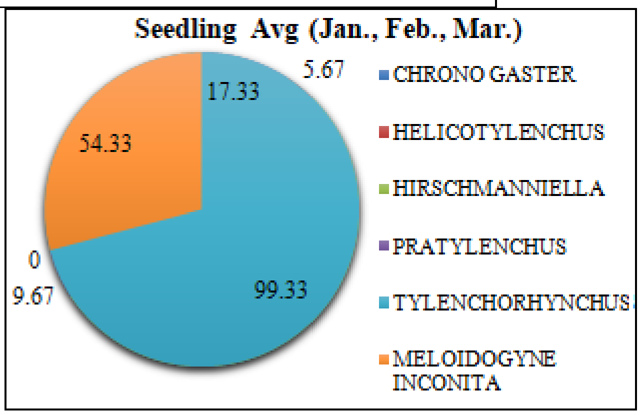
Crop stage	Period	*C. gracilis*	*H. indicus*	*H. oryzae*	*P. elamini*	*T. nudus*	*M. inconita*	Total
Seeding	Oct	23	6	180	14		64	357
	Nov	20	9	116	14		42	201
	Dec	14	4	82	4		34	138
	Avg	19	6.33	126	10.67		46.67	232
Seedling	Jan	8	2	38	6		18	72
	Feb	7	7	66	7		42	129
	Mar	18	5	24	10		0	57
	Avg	11	4.67	42.67	7.67		20	86
Grand Growth	Apr	22	6	102	12		61	203
	May	17	6	83	7		24	137
	Jun	14	7	40	5		12	78
	Avg	17.67	6.33	75	8		32.33	139.3
Post Harvest	Jul	18	3	110	6		38	175
	Aug	27	8	148	7		64	254
	Sep	32	10	156	16		72	286
	Avg	25.67	7	138	9.67		58	238.33

Table 7.5: Control of Nematodes in Makhana Cultivated Ponds Through Various Herbal Treatments

Treatments	Active ingredients	Dose	No. of Nematodes per 100g of Soil								% Decrease Over Control			
			Control				After Treatment of 21 Days							
			Jan	May	Sep	Avg	Jan	May	Sep	Avg	Jan	May	Sep	Avg
1. Neem leaves (*Azadirach-taindica*)		20 kg/400m^2	968	1248	1630	1282	424	618	892	644.7	56.2	50.2	453	50.7
2. Papaya leaves (*Carica papaya*)		20 kg/400m^2	756	1061	1580	1132.30	495	845	1354	898	34.5	50.4	14.4	33.1
3. Kaner Leaves (*Thevetiaperuviana*)		20 kg/400m^2	622	976	1334	977.30	436	600	968	688	30.2	32.4	27.5	30.0
4. Giloy Leaves (*Tinosporacordifolia*)		20 kg/400m^2	562	824	1254	880	347	511	865	574.3	38.3	38.0	31.0	35.7
5. Castor Leaves (*Ricinus communis*)		20 kg/400m^2	918	1126	1462	1169	496	631	905	677.3	46.0	46.1	38.1	43.4







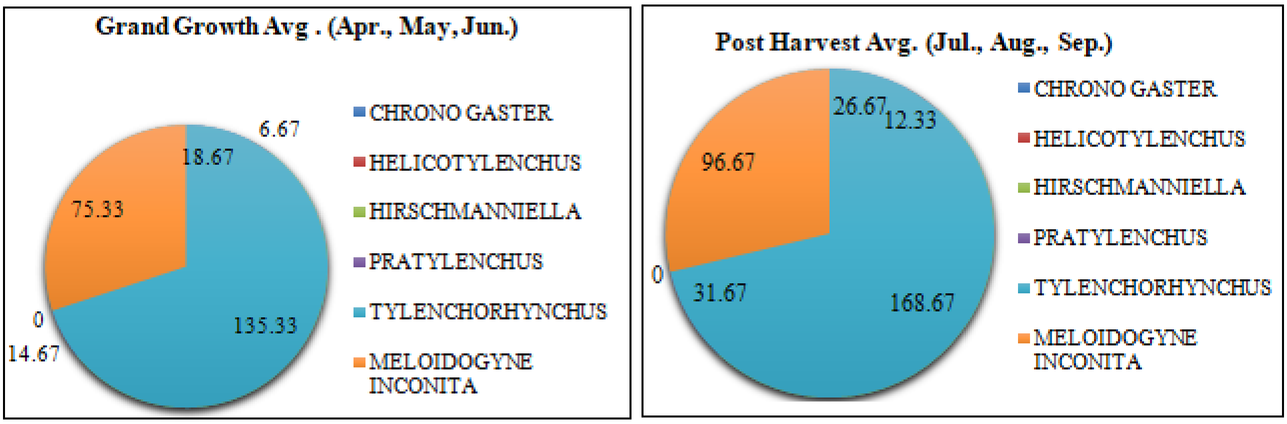
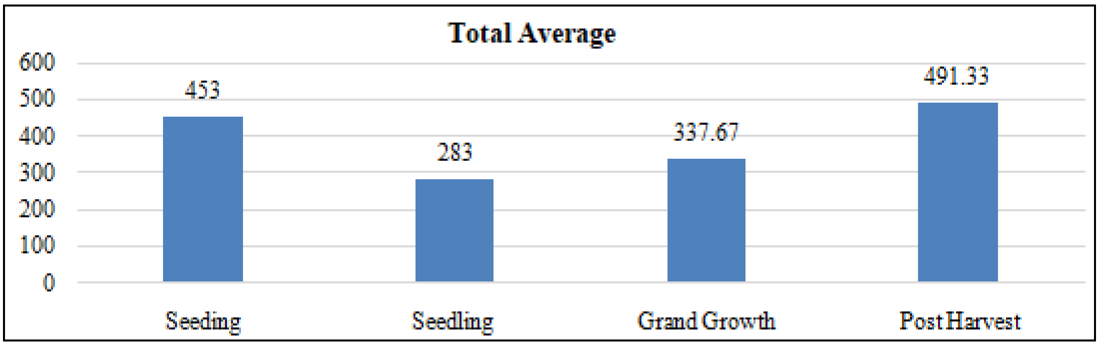


Figure 7.2: Average Population Density (per 100g Soil) and Seaosnal Fluctuations of Different Nematodes around the Rhizospheres of Makhana in Different Stages of Growth at Site - I



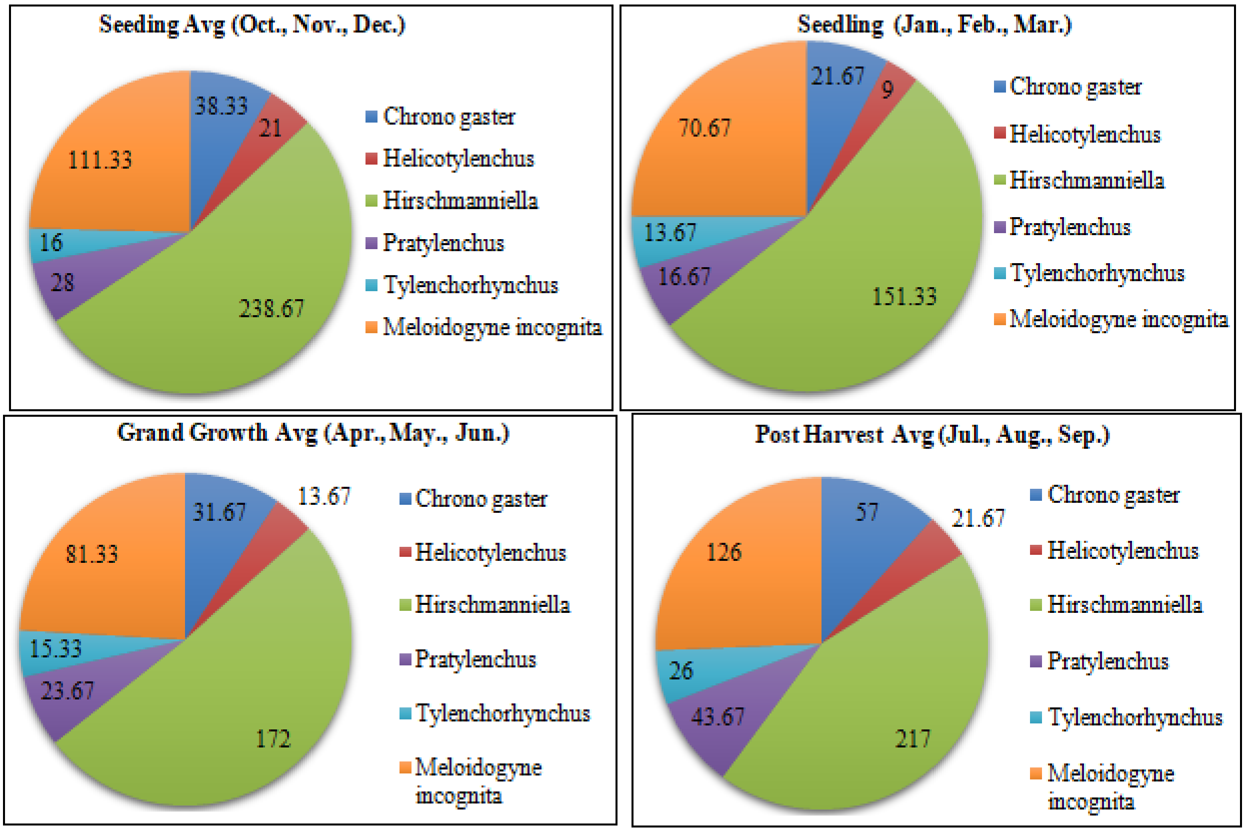


Figure 7.3: Average Population Density (per 100g Soil) and Seaosnal Fluctuations of DifferentNematodes Around the Rhizospheres of Makhana in Different Stages of Growth at Site -II

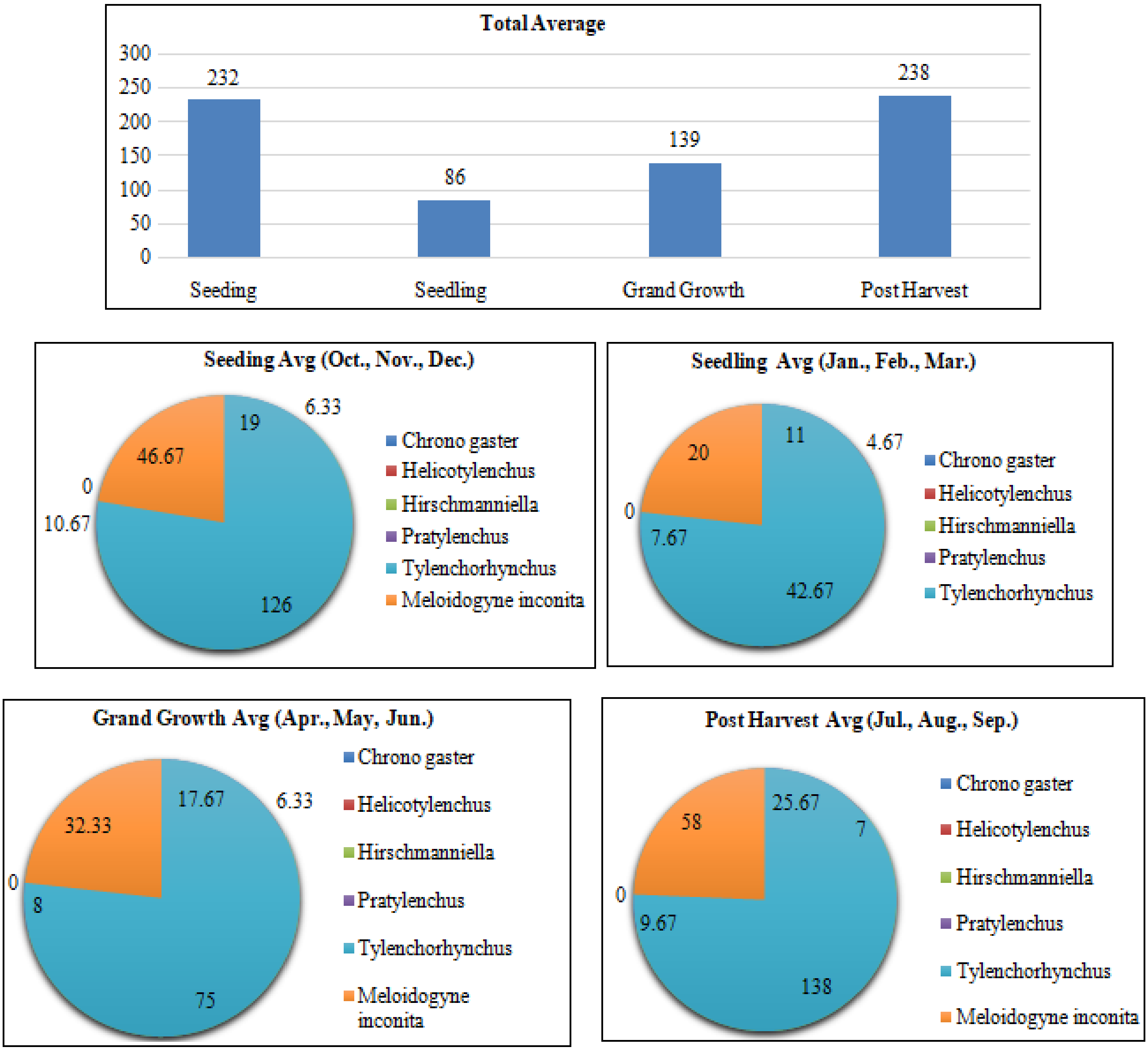


Figure 7.4: Average Population Density (per 100g Soil) and Seaosnal Fluctuations of Different Nematodes around the Rhizospheres of Makhana in Different Stages of Growth at Site -III

4. Discussion

Plant parasitic nematodes cause severe crop losses. *Hirschmanniella oryzae* causes **root necrosis** disease in makhana plant [25] and is responsible for appreciable loss in makhana yield by damaging root hairs and causing necrosis. This nematode is also a serious pathogen of rice plant causing root necrosis disease and heavy loss in rice yield by damaging root hairs [26, 27]. Species of *Hirschmanniella* have been considered as one of the most important plant parasitic nematode of submerged as well immersed vascular aquaphytes [28]. Presence of high population of *H. oryzae* around the rhizosphere and a few inside roots of makhana in both mesotrophic and eutrophic ponds clearly indicates that this nematode is an important pathogen of this crop and appear to be serious threat to its cultivation [31] Similarly, the presence of other parasitic nematodes like *Pratylenchus elamini* cause **lesion** disease and *Tylenchorhynchus nudus* cause **dwarfing** disease [29] in makhana crop either singly or synergistically.

Hirschmanniella oryzae dominated among the parasitic forms and non- parasitic forms like *Chronogaster gracilis* in non-parasitic forms. Total population of nematodes as well as population of individual nematode species was found to be much higher in mesotrophic pond in comparison to eutrophic pond. *Tylenchorhynchus nudus* was not found in eutrophic pond [9, 30, 31]. The presence of more organic matter in eutrophic ponds and their decomposition products make the environment less congenial to nematode multiplication resulting in low population density in comparison to mesotrophic pond [32].

High population of *Hirschmanniella oryzae* around rhizosphere of makhana and presence of a few individuals inside the root suggests that this crop is a good host of *H. oryzae* [31]. Gerber and Smart (1987) reported the species of *Hirschmanniella* along with other nematodes being the parasite of several vascular aquatic plants [28]. A strong seasonal variation in nematode population of both mesotrophic and eutrophic ponds clearly indicate the impact of season [31].The population of nematode remained high during rainy season and early summer due to favourable temperatures and availability of feeder roots. During winter (December to February) and late summer (May- June) the population of nematodes is low due to unfavourable temperatures. Several workers have observed the important roles of temperature as well host plants in population fluctuations of several nematodes.

Practically no farmers were found to practice nematode management during makhana cultivation due to the economic reasons *i.e.* high cost of nematicides, labour problems & difficulties. However, less no. of nematodes in pond soil around rhizosphere of makhana were found in integrated fish carp culture with makhana cultivation. This may be due to consumption of these nematodes by fishes in integrated fish makhana cultivation [33]. However, application of dried leaves of neem (kg/ha) was found to reduce up to 40% of the population of nematodes in makhana cultivated ponds. Rotation of makhana cultivation and fish culture may be another option to reduce nematode population in makhana cultivated ponds [34].

Soil treatment with nematicides before seeding stage may also be practiced to control nematodes during cultivation of makhana but it may affect the population of fish in integrated culture because nematodes are one of the foods of fishes in the ponds [33]. More cost effective, organic control measures such as dry fallow and rotation may prove effective, but farmers cannot afford taking land out of production for the necessary length of time to abode the destructive levels of root nematodes [35].

Weed control and natural resistance of makhana may prove effective like rice in the future [27] but the lack of extensive research effects for varietal resistance renders the latter unsuccessful. Fortunately, nutritional experiments indicate that when plants are given the proper culture care they can produce a satisfactory crop which may allow a level of tolerance of makhana like rice to root nematodes [27].

5. Conclusions

Control of nematodes especially in aquatic agricultural fields is a serious problem for farmers. From economic point of view it is very difficult to practice nematode management by the farmers during makhana cultivation due to higher costs of nematicides, labour problems and other difficulties. Soil treatment with nematicides before seeding stage may also be practiced to control nematodes during cultivation of makhana but it may affect the production of fish in integrated culture because nematodes are one of the foods of fishes in the ponds as reported earlier. However, application of dried leaves of neem and castor may be helpful in reducing the population of nematodes in makhana cultivated ponds. This will not cause damage like nematicides to fishes and other small aquatic speciesand will be helpful in increasing the yield of the crop.

6. Acknowledgement

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